

CLAIMS

What is claimed is:

1. A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule,
5 said binding domain polypeptide comprising a heavy chain variable region, said heavy chain variable region comprising an amino acid substitution or deletion at one or more amino acid residues; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second
10 polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.
2. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv.
3. A protein of claim 1 wherein the one or more amino acid substitution
15 or deletion in said heavy chain variable region is effective to increase expression or stability of said protein relative to a protein without said deletion or substitution.
4. A protein of claim 1 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.
- 20 5. A protein of claim 4 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.
6. A protein of claim 5 wherein the first target molecule and the second
25 target molecule are different.
7. A protein of claim 5 wherein the first target molecule and the second target molecule are the same.
8. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising one or more amino acid substitution in positions 9, 10, 11, 12,
30 108, 110, 112 in said heavy chain variable region.

9. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising a an amino acid substitution at position 11 in said heavy chain variable region.
10. A protein of claim 9 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.
11. A protein of claim 9 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, and glutamine.
12. A protein of claim 9 where leucine is replaced by serine at position 11.
13. A protein of claim 9 where leucine is replaced by des-leucine at position 11.
14. A protein of claim 12 having an increased recombinant expression or stability relative to said protein not having an amino acid substitution at position 11.
15. A protein of claim 14 wherein the expression of said protein having an amino acid substitution at position 11 is 10-100 fold greater than said protein without a substitution at position 11.
16. A protein of claim 14 wherein said expression is in mammalian cells.
17. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.
18. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and said binding domain polypeptide comprises a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.
19. A protein of claim 18 wherein the amino acid at position 106 has been substituted or deleted.

20. A protein of claim 2 wherein said binding domain polypeptide binds to a tumor antigen.
21. A protein of claim 2 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.
- 5 22. A protein of claim 2 wherein said binding domain polypeptide binds to a cancer cell antigen.
23. A protein of claim 22 wherein said cancer cell antigen is a surface antigen.
24. A protein of claim 22 wherein said cancer cell antigen is an
10 intracellular antigen.
25. A protein of claim 1 wherein said binding domain polypeptide binds to a B cell antigen.
26. A protein of claim 25 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
- 15 27. A protein of claim 2 wherein said single chain Fv binds to a B cell antigen.
28. A protein of claim 27 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
29. A protein of claim 28 wherein said single chain Fv is selected from
20 the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.
30. A protein of claim 2 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.
- 25 31. A protein of claim 1 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.
32. A protein of claim 31 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.
33. A protein of claim 1 wherein said binding domain polypeptide binds
30 to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10,

CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

5 34. A protein of claim 1 wherein said protein is capable of forming a complex comprising two or more of said proteins.

 35. A protein of claim 34 wherein said complex is a dimer.

 36. A protein of claim 1 wherein said protein is a monomer.

 37. A protein of claim 1 coupled to a drug, toxin, immunomodulator,
10 polypeptide effector, isotope, label, or effector moiety.

 38. A protein of claim 1 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular
15 activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

 39. A protein of claim 38 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other
20 second messengers, ion channels, and secretory pathway components.

 40. A protein of claim 38 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

 41. A protein of claim 40 wherein said induction of one or more immune
25 effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

 42. A protein of claim 38 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity.

 43. A protein of claim 42 wherein said cellular transcriptional activity is
30 increased.

44. A protein of claim 42 wherein said cellular transcriptional activity is decreased.
45. A protein of claim 38 wherein said one or more biologically active molecules is a protease.
- 5 46. A protein of claim 38 wherein said one or more biologically active molecules is a cytokine.
47. A protein of claim 46 wherein said cytokine is selected from the group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.
- 10 48. A protein of claim 38 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.
49. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.
50. A protein of claim 38 which is capable of neutralization of a toxin,
15 wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.
51. A protein of claim 38 which is capable of neutralization of a toxin,
20 wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.
52. A protein of claim 1 wherein said protein is capable of binding to an intracellular target to effect a cellular function.
- 25 53. A protein of claim 1 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser.
54. A protein of claim 53 comprising three Gly-Gly-Gly-Gly-Ser
30 peptides.

55. A protein of claim 1 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

56. A protein of claim 1 wherein said binding domain polypeptide
5 comprises a humanized immunoglobulin variable region.

57. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

10 58. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

59. A protein of claim 2 wherein said N-terminally truncated
15 immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

60. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2
20 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

61. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

62. A protein of claim 2 wherein said binding domain polypeptide is a
25 single chain Fv that comprises at least a portion of a human constant region.

63. A protein of claim 1 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof,

IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

64. A protein of claim 1 wherein said connecting region comprises a human IgE hinge or a portion thereof.

5 65. A protein of claim 1 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

66. A protein of claim 1 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

10 67. A protein of claim 1 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

68. A protein of claim 1 wherein said connecting region comprises a glycosylation site.

69. A protein of claim 1 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

15 70. A protein of claim 1 wherein said connecting region has one cysteine residue.

71. A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

20 72. A protein of claim 1 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

73. A protein of claim 1 where said connecting region comprises three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted and said proline residue is substituted or deleted.

25 74. A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is substituted or deleted.

75. A protein of claim 74 wherein said wild-type hinge region polypeptide is from human IgG1.

76. A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region comprising one or more amino acid deletion or substitution in positions 9, 10, 11, 12, 108, 110, 112; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

77. A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine is replaced by serine at position 11 in the first framework region of the heavy chain variable region; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

78. A protein of claim 77 further comprising a substitution or deletion of the amino acid at position 10 in the first framework region of the heavy chain variable region.

79. A protein of claim 77 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

80. A protein of claim 77 wherein the numbering of the residues is the EU index according to Kabat.

81. A protein of claim 77 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-

terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

82. A protein of claim 77 wherein said connecting region comprises an IgA hinge region or portion thereof.

5 83. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine in the connecting region, and said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

10 84. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a murine IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from murine IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

15 85. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein said first, second, and third cysteine residues in the connecting region are replaced by serine and said proline residue in the connecting region is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein lysine is replaced by serine at position 322 in said CH2 region.

20 86. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein lysine is replaced by serine at position 322 in said CH2 region.

25 87. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 331 in said CH2 region.

88. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
5 from IgG₁ wherein proline is replaced by serine at position 331 in said CH2 region.

89. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁
10 wherein threonine is replaced by asparagine at position 256 in said CH2 region.

90. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains
15 from IgG₁ where in the CH2 domain arginine is replaced by glutamine at position 255, threonine is replaced by asparagine at position 256, proline is replaced by alanine at position 257, and glutamic acid is replaced by lysine at position 258.

91. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said
20 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein lysine is replaced by glutamine at position 290 in said CH2 region.

92. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein alanine is replaced by proline at position 339 in said CH2 region.

93. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region
30 said first, second, and third cysteine residues are replaced by serine and said proline residue

is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG1.

94. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region
5 said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

95. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region
10 said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

96. A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region
15 comprising CH2 and CH3 domains from IgG₁.

97. A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a FC2-2 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
20 from IgG₁.

98. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a UCHL-1 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
25 from IgG₁.

99. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 5B9 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3
30 domains from IgG₁.

100. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
5 from IgG₁.

101. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3 domains from
10 IgG₁.

102. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
15 from IgG₁.

103. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma comprising a connecting region wherein said third cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

20 104. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

25 105. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a murine IgA hinge region, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

106. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a human IgA hinge region and said heavy chain constant region comprises CH2 and CH3 domains from human IgA, where said CH3 comprises a deletion or substitution in
5 four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

107. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is
10 replaced by serine, and said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

108. A protein of claim 81 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, and
15 said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

109. A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region where leucine is replaced by serine at position 11 in the first framework region of the heavy chain
20 variable region; wherein said protein has an increased expression or stability in mammalian cells relative to a protein not having said amino acid substitution; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-
25 chain Fv protein is capable of at least one immunological activity.

110. A non-naturally occurring single chain Fv protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine
30 at is replaced by serine at position 11 in the first framework region of the heavy chain variable region; and ii) a second polypeptide comprising an N-terminally truncated IgE

immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

111. A protein of claim 110 wherein the amino acid substitution at position 5 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

112. A protein of claim 110 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from IgE.

10 113. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE.

114. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant 15 region comprises CH2, CH3, and CH4 domains from murine IgE.

115. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from human IgE.

116. A non-naturally occurring single chain protein, including a first 20 polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first 25 cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

117. A protein of claim 116 wherein said binding domain polypeptide is a 30 single chain Fv.

118. A protein of claim 116 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

5 119. A protein of claim 118 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

120. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable
10 region has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, 112.

121. A protein of claim 117 wherein said binding domain polypeptide is a single chain Fv comprising a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12,
15 80, 81, 83, 105, 106, and 107.

122. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.

123. A protein of claim 122 wherein acid at position of 11 of the single
20 chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

124. A protein of claim 122 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected
25 from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, and glutamine.

125. A protein of claim 122 wherein leucine is replaced by serine at position 11.

126. A protein of claim 122 wherein leucine is replaced by des-leucine at position 11.

127. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.

5 128. A protein of claim 121 where leucine is replaced by serine at position 106.

129. A protein of claim 117 wherein said binding domain polypeptide binds to a tumor antigen.

130. A protein of claim 117 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.

10 131. A protein of claim 117 wherein said binding domain polypeptide binds to a cancer cell antigen.

132. A protein of claim 131 wherein said cancer cell antigen is a surface antigen.

15 133. A protein of claim 131 wherein said cancer cell antigen is an intracellular antigen.

134. A protein of claim 116 wherein said binding domain polypeptide binds to a B cell antigen.

135. A protein of claim 135 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

20 136. A protein of claim 117 wherein said single chain Fv binds to a B cell antigen.

137. A protein of claim 136 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

25 138. A protein of claim 137 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

139. A protein of claim 117 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

140. A protein of claim 116 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

141. A protein of claim 136 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.

5 142. A protein of claim 116 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand),
10 ICOS, L6, B7-H1, and HLA class II.

143. A protein of claim 116 wherein said protein is capable of forming a complex comprising two or more of said proteins.

144. A protein of claim 143 wherein said complex is a dimer.

145. A protein of claim 116 wherein said protein is a monomer.

15 146. A protein of claim 116 coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety.

147. A protein of claim 116 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active
20 signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

148. A protein of claim 147 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group
25 consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.

149. A protein of claim 147 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

150. A protein of claim 149 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

5 151. A protein of claim 147 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity.

152. A protein of claim 151 wherein said cellular transcriptional activity is increased.

153. A protein of claim 151 wherein said cellular transcriptional activity is decreased.

10 154. A protein of claim 147 wherein said one or more biologically active molecules is a protease.

155. A protein of claim 147 wherein said one or more biologically active molecules is a cytokine.

15 156. A protein of claim 155 wherein said cytokine is selected from the group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

157. A protein of claim 147 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

20 158. A protein of claim 147 A protein which is capable of neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

25 159. A protein of claim 147 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.

30 160. A protein of claim 147 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

161. A protein of claim 116 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser.

5 162. A protein of claim 116 comprising three Gly-Gly-Gly-Gly-Ser peptides.

163. A protein of claim 116 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

10 164. A protein of claim 116 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

165. A protein of claim 116 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

15 166. A protein of claim 116 wherein said immunoglobulin heavy chain constant region polypeptide is without a functionally active CH1 domain.

167. A protein of claim 116 said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

20 168. A protein of claim 116 comprising at least a portion of a human constant region.

169. A protein of claim 116 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

170. A protein of claim 116 wherein said connecting region comprises a human IgE hinge or a portion thereof.

171. A protein of claim 116 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

5 172. A protein of claim 116 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

173. A protein of claim 116 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

10 174. A protein of claim 116 wherein said connecting region comprises a glycosylation site.

175. A protein of claim 116 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

176. A protein of claim 116 wherein said connecting region has one cysteine residue.

15 177. A protein of claim 116 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

178. A protein of claim 116 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

20 179. A protein of claim 116 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

25 180. A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 domain.

181. A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 domains from IgG₁.

182. A protein of claim 179 where said single chain protein comprises a
5 FC2-2 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

183. A protein of claim 179 where said single chain protein comprises a
10 UCHL-1 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

184. A protein of claim 179 where said single chain protein comprises a
15 5B9 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

185. A protein of claim 179 where said single chain protein comprises a
20 2H7 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

186. A protein of claim 179 where said single chain protein comprises a
25 2H7 single chain Fv binding domain, wherein in the connecting region said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

187. A protein of claim 179 where said single chain protein comprises a
30 2H7 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein phenylalanine is replaced by tyrosine at position 405 in said CH3 region.

188. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises CH2 and CH3 domains from IgG₁
5 wherein phenylalanine is replaced by alanine at position 405 in said CH3 region.

189. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁
10 wherein tyrosine is replaced by alanine at position 407 in said CH3 region.

190. A protein of claim 189 having an apparent molecular weight of about 75 kDa.

191. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second,
15 and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407 in said CH3 region.

192. A protein of claim 179 where said single chain protein comprises a
20 2H7 single chain Fv binding domain, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

193. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and third
25 cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

194. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and second
30 cysteine residues are replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

195. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.
- 5 196. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said third cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.
- 10 197. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first cysteine residue is replaced by serine, and where said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains.
- 15 198. A protein of claim 179 where said single chain protein comprises a HD37 single chain Fv binding domain, wherein in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.
- 20 199. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG₁.
200. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG₂.
- 25 201. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG₃.
- 30 202. A protein of claim 179 where said single chain protein comprises a CD-16-6 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region.

203. A protein of claim 179 where said single chain protein comprises a CD-16 receptor binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein
5 proline is replaced by serine at position 238 in said CH2 region.

204. A protein of claim 179 where said single chain protein comprises a 2e12 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁
10 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

205. A protein of claim 179 where said single chain protein comprises a 10A8 scFv single chain Fv binding domain, wherein in the connecting region said first,
15 second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

206. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 40.2.36 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and
25 wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

207. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
30 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

208. A protein of claim 179 where said single chain protein comprises a
5 single chain Fv binding domain from a G19-4 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and
10 cytoplasmic tail domains.

209. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a G19-4 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

15 210. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising CD80
20 transmembrane and cytoplasmic tail domains.

211. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
25 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

212. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said
30 first, second and third cysteine residues are replaced by serine and said proline residue is

replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

213. A protein of claim 179 where said single chain protein comprises a
5 single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

10 214. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said
15 CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

215. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
20 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

216. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

217. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
5 from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

218. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
10 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

219. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
15 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

220. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
20 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.
25

221. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein said CH3 domain is attached to a
30 polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

222. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
5 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

223. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 scFv hybridoma, where in the connecting region
10 said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

224. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said
15 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and
20 cytoplasmic tail domains.

225. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
25 from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

226. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is
30 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

227. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said
5 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

10 228. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising mcasp8
15 transmembrane and cytoplasmic tail domains.

229. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains
20 from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

230. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

231. A protein of claim 179 where said single chain protein comprises a
30 single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said

first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcas3 transmembrane and cytoplasmic tail domains.

232. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁, wherein said CH3 domain is attached to a polypeptide comprising hcas8 transmembrane and cytoplasmic tail domains.

233. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁ wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcas8 transmembrane and cytoplasmic tail domains.

234. A protein of claim 179 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

235. A protein of claim 179 where said single chain protein comprises a G28-1 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises G28-1 single chain Fv CH2 and CH3 domains.

236. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD comprising the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell

population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

237. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD consisting essentially of the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

238. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

239. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution in positions 12, 80, 81, 83, 105, 106, and 107; ii) a

second polypeptide comprising a connecting region attached to said first polypeptide; and
iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain
constant region polypeptide attached to the second polypeptide, wherein said non-naturally
occurring single-chain protein is capable of at least one immunological activity.

5 240. A protein of claim 238 wherein the one or more amino acid
substitution or deletion in said light chain variable region is effective to increase expression
or stability of said protein relative to a protein without said deletion or substitution.

 241. A protein of claim 238 wherein said binding domain polypeptide is a
single chain Fv.

10 242. A protein of claim 238 wherein said binding domain polypeptide
comprises an immunoglobulin light chain variable region polypeptide and an
immunoglobulin heavy chain variable region polypeptide.

 243. A protein of claim 242 further comprising a second binding domain
polypeptide capable of binding a second target molecule, said second binding domain
15 polypeptide comprising an immunoglobulin light chain variable region polypeptide and an
immunoglobulin heavy chain variable region polypeptide.

 244. A protein of claim 241 wherein said binding domain polypeptide is a
single chain Fv and said light chain variable region of said single chain Fv has an amino acid
deletion or substitution at position 106.

20 245. A protein of claim 241 wherein said binding domain polypeptide is a
single chain Fv and the amino acid at position 106 of the heavy chain variable region of said
single chain Fv has been deleted.

 246. A protein of claim 238 wherein said binding domain polypeptide is a
single chain Fv and said light chain variable region of said single chain Fv has an amino acid
25 substitution at position 106.

 247. A protein of claim 244 wherein leucine is replaced by serine at
position of 106.

 248. A protein of claim 244 wherein leucine is replaced by des-leucine at
position of 106.

249. A protein of claim 244 wherein the amino acid substituted for the amino acid at position 106 of the single chain Fv light chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.
- 5 250. A protein of claim 244 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.
251. A protein of claim 241 wherein said binding domain polypeptide binds to a tumor antigen.
- 10 252. A protein of claim 241 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.
253. A protein of claim 241 wherein said binding domain polypeptide binds to a cancer cell antigen.
254. A protein of claim 253 wherein said cancer cell antigen is a surface
15 antigen.
255. A protein of claim 253 wherein said cancer cell antigen is an intracellular antigen.
256. A protein of claim 238 wherein said binding domain polypeptide binds to a B cell antigen.
- 20 257. A protein of claim 256 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
258. A protein of claim 241 wherein said single chain Fv binds to a B cell antigen.
259. A protein of claim 258 wherein said B cell antigen is selected from
25 the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
260. A protein of claim 259 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.
261. A protein of claim 238 wherein said binding domain polypeptide is an
30 scFv that binds to a B cell differentiation antigen.

262. A protein of claim 261 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.
263. A protein of claim 238 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.
264. A protein of claim 238 wherein said protein is capable of forming a complex comprising two or more of said proteins.
265. A protein of claim 264 wherein said complex is a dimer.
266. A protein of claim 238 wherein said protein is a monomer.
267. A protein of claim 238 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.
268. A protein of claim 267 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.
269. A protein of claim 267 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.
270. A protein of claim 267 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.
271. A protein of claim 267 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity.

272. A protein of claim 271 wherein said cellular transcriptional activity is increased.
273. A protein of claim 271 wherein said cellular transcriptional activity is decreased.
- 5 274. A protein of claim 267 wherein said one or more biologically active molecules is a protease.
275. A protein of claim 267 wherein said one or more biologically active molecules is a cytokine.
276. A protein of claim 275 wherein said cytokine is selected from the
10 group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.
277. A protein of claim 267 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.
278. A protein of claim 267 which is capable of neutralization of a toxin,
15 wherein said toxin is selected from the group consisting of endotoxins and exotoxins.
279. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin,
20 *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.
280. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.
- 25 281. A protein of claim 238 wherein said protein is capable of binding to an intracellular target to effect a cellular function.
282. A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region
30 polypeptide.

283. A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.
- 5 284. A protein of claim 238 comprising at least a portion of a human constant region.
285. A protein of claim 238 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.
- 10 286. A protein of claim 238 wherein said connecting region comprises a human IgE hinge or a portion thereof.
- 15 287. A protein of claim 238 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.
288. A protein of claim 238 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.
289. A protein of claim 238 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.
- 20 290. A protein of claim 238 wherein said connecting region comprises a glycosylation site.
291. A protein of claim 238 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.
- 25 292. A protein of claim 238 wherein said connecting region has one cysteine residue.
293. A protein of claim 238 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

294. A protein of claim 238 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

295. A protein of claim 238 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

296. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprises at least a portion of an IgA hinge region, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

297. A protein of claim 296 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

298. A protein of claim 297 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

299. A protein of claim 296 wherein said connecting region consist essentially of at least a portion of an IgA hinge region.

300. A protein of claim 296 wherein said connecting region consist essentially of an IgA hinge region.

301. A protein of claim 300 that is expressed as a protein having an apparent molecular of at least about 70 kDa.

302. A protein of claim 300 that is expressed as a protein having an apparent molecular of between about 70 kDa and about 700 kDa.

303. A protein of claim 296 wherein said connecting region comprises between 10 to 50 amino acids.

304. A protein of claim 296 wherein said connecting region comprises a modified human IgA immunoglobulin hinge region polypeptide.
305. A protein of claim 296 wherein said connecting region has only one cysteine residue.
- 5 306. A protein of claim 296 wherein said connecting region two cysteine residues.
307. A protein of claim 296 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide in the third polypeptide comprises IgA immunoglobulin heavy chain constant regions.
- 10 308. A protein of claim 307 comprising CH2 and CH3 domains from IgA immunoglobulin heavy chain constant regions.
309. A protein of claim 308 wherein said IgA CH3 constant region domain comprises a substitution or deletion in one or more amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.
- 15 310. A protein of claim 308 wherein said IgA heavy chain constant region is capable of associating with a J chain polypeptide.
311. A protein of claim 309 wherein said IgA CH3 region domain comprises a deletion in between four and twenty amino acids.
312. A protein of claim 309 wherein said IgA region domain comprises a
20 deletion of four amino acids.
313. A protein of claim 309 wherein said IgA region domain comprises a deletion of twenty amino acids.
314. A protein of claim 296 wherein said single chain protein comprises a L6 single chain Fv binding domain, wherein the connecting region comprises an IgA hinge
25 region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.
315. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a L6 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region
30 comprising CH2 and CH3 domains from IgG₁.

316. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

5 317. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of
10 associating with a J chain polypeptide.

318. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

15 319. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in eighteen amino acids that render the IgA heavy chain constant region incapable of associating with a J chain
20 polypeptide.

320. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino
25 acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

321. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a
30 IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3

domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

5 322. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, said connecting region comprises a IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain
10 polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

 323. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region
15 comprising CH2 and CH3 domains from IgG₁.

 324. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG₁.

20 325. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of
25 associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

 326. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region

comprises CH2 and CH3 domains from IgA, wherein the IgA heavy chain constant region is capable of associating with a J chain polypeptide.

327. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, wherein the connecting region
5 comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA.

328. A non-naturally occurring single chain protein comprising i) a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, and ii) a second polypeptide comprising at least a portion of an IgE
10 immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

329. A protein of claim 328 wherein said binding domain polypeptide comprises a non-IgE variable region.

15 330. A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

331. A protein of claim 328 wherein said binding domain polypeptide
20 comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

332. A protein of claim 331 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an
25 immunoglobulin heavy chain variable region polypeptide.

333. A protein of claim 332 wherein said immunoglobulin light and heavy chains comprise IgG immunoglobulin light and heavy chains or a portions thereof.

334. A protein of claim 328 wherein said binding domain polypeptide comprises at least a portion of an IgG immunoglobulin variable region.

335. A protein of claim 334 wherein said IgG variable region comprises a heavy chain variable region and a light chain variable region.
336. A protein of claim 328 that is capable of binding to a Fc receptor specific for IgE.
- 5 337. A protein of claim 328 that does not have IgE variable chain region.
338. A protein of claim 328 wherein the binding domain consist essentially of an IgG immunoglobulin light chain region and a IgG immunoglobulin light and heavy chain region.
339. A protein of claim 328 wherein said IgE immunoglobulin heavy chain
10 constant region polypeptide comprises CH2 and CH3 domains.
340. A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2, CH3 and CH4 domains.
341. A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable
15 region has an amino acid substitution at amino acid position 11.
342. A protein of claim 328 wherein said single chain protein is a single chain Fv selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.
343. A protein of claim 328 wherein said single chain protein is a single
20 chain Fv comprising a 2H7 single chain Fv or a G28-1 single chain Fv.
344. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a non-naturally occurring single chain protein, said protein comprising a first polypeptide comprising a binding domain polypeptide, said binding domain polypeptide comprising at least a portion of an
25 IgG variable region and being capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

345. The method of claim 344 wherein said protein is administered into the blood stream of a patient.

346. The method of claim 344 wherein monocytes are activated but mast cells are not activated as a result of administration of the protein.

5 347. The method of claim 344 wherein the protein is administered or coadministered with a histamine release blocker.

348. A method of depleting cells in an animal comprising administering a modified IgE protein into the blood stream of an animal.

10 349. The method of claim 348 wherein the modified IgE protein is administered or coadministered with a histamine release blocker.

350. A protein of claim 328 wherein said connecting region comprises a IgG hinge or portion thereof, and said heavy chain constant region is from IgE and comprises CH3 and CH4 domains without a CH2 domain.

15 351. A protein of claim 328 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12, and said heavy chain constant region comprises IgE CH2, CH3, and CH4 domains, and wherein said heavy chain constant region is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

20 352. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; ii) a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprising three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-
25 chain protein is capable of at least one immunological activity.

353. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not substituted or deleted, and where said second and third
30 cysteine are substituted or deleted.

354. A protein of claim 353 wherein said second and third cysteine residues are substituted by another amino acid.

355. A protein of claim 354 wherein said second and third cysteine residues are substituted by serine residues.

5 356. A protein of claim 352 that is expressed as a homogeneous protein having an apparent molecular of about 75 kDa.

357. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein
10 said first cysteine residue is substituted or deleted.

358. A protein of claim 352 where said second and third cysteine residues in said connecting region are not substituted or deleted.

359. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to
15 said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said second cysteine residue is substituted or deleted.

360. A protein of claim 352 wherein said first and third cysteine residues in said connecting region are not substituted or deleted.

361. A protein of claim 352 wherein said connecting region comprises
20 first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said third cysteine residue is substituted or deleted.

362. The protein of claim 10 where said first and second cysteine residues in said connecting region are not substituted or deleted.

25 363. A protein of claim 352 wherein said connecting region comprises between 5 and 65 amino acids.

364. A protein of claim 352 wherein said connecting region comprises between 10 and 50 amino acids.

365. A protein of claim 352 wherein said connecting region comprises
30 between 15 and 35 amino acids.

366. A protein of claim 352 wherein said connecting region comprises at least five consecutive hinge region amino acids.

367. A protein of claim 352 wherein said connecting region comprises at least ten consecutive hinge region amino acids.

5 368. A protein of claim 352 wherein said connecting region comprises at least fifteen consecutive hinge region amino acids.

369. A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG₁ hinge region.

10 370. A protein of claim 369 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₁ hinge region.

371. A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG₂ hinge region.

15 372. A protein of claim 371 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₂ hinge region.

373. A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG₃ hinge region.

20 374. A protein of claim 373 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₃ hinge region.

375. A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG₄ hinge region.

25 376. A protein of claim 375 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG₄ hinge region.

377. A protein of claim 352 wherein said second and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is

replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

378. A protein of claim 352 wherein said first and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said
5 heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

379. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target
10 molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal
15 to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

380. A protein of claim 352 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide.

20 381. A protein of claim 352 wherein said connecting region has only one cysteine residue.

382. A protein of claim 381 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide.

383. A protein of claim 352 wherein said N-terminally truncated
25 immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

384. A protein of claim 352 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2

constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

385. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion that inhibits two of said proteins from associating together to form a non covalent dimer, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

386. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion at positions 405 and 407, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

387. A protein of claim 252 or 253 comprising amino acid substitutions at position 405 and position 407.

388. A protein of claim 387 wherein phenylalanine is replaced with alanine at position 405 and tyrosine is substituted with alanine at position 407.

389. A protein of claim 387 having an apparent molecular weight of between about 40 kDa and about 50 kDa.

390. A protein of claim 387 that has a biological activity of an agonist or antagonist.

391. A protein of claim 390 that has a biological activity of an antagonist.

392. A protein of claim 387 wherein only one amino acid at positions 405 and 407 is substituted.

393. A protein of claim 392 having an apparent molecular weight of between about 70 kDa and about 80 kDa.

394. A protein of claim 387 where phenylalanine is replaced by alanine at position 405.

5 395. A protein of claim 387 where phenylalanine is replaced by tyrosine at position 405.

396. A protein of claim 386 where tyrosine is replaced by alanine at position 407.

10 397. A protein of claim 386 comprising amino acid substitutions at position 405 and position 407.

398. A protein of claim 393 wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407.

399. A polynucleotide that encodes a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109.

15 400. A polynucleotide that encodes a protein according to any one of claims 116, 120, 121, and 180-235.

401. A polynucleotide that encodes a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386.

20 402. A polynucleotide of any one of claims 399-401 that is operably linked to a promoter or other sequence that enhances expression of the polynucleotide in a cell.

403. A cell containing a polynucleotide of any one of claims 399-401.

404. A recombinant vector capable of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109.

25 405. A recombinant vector capable of expressing a protein according to any one of claims 116, 120, 121, and 180-235.

406. A method of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109 under conditions in which the protein is expressed.

407. A method of expressing a protein according to any one of claims 116, 120, 121, and 180-235 under conditions in which the protein is expressed.

408. A method of expressing a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 under conditions in which the protein is expressed.

409. A composition comprising a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 in combination with one or more additional
5 therapeutic compounds.

410. A method of treating a subject comprising the steps of administering to said subject a one or more first compound according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386, administering or coadministering a second compound to the subject in combination said first compound, wherein the treatment is effective to treat or
10 prevent a disease, ailment, or undesired condition.

411. The method according to claim 410 wherein the second compound is selected from the group consisting of a chemotherapeutic compound, therapeutic drug, angiogenesis inhibitors, steroids, B-cell activators, T-cell activators, colony stimulating factors, tumor necrosis factors, interferons, antibody, binding construct of the invention,
15 gene therapy, retinoids, surgical procedures, alkylating agents, nucleoside analogues, topoisomerase II, VEGF, IFN-alpha, DMAR agents, interleukin-1, glucocorticoids, p38 inhibitors, interleukin-4, interleukin-6, interleukin-2, interleukin-12, IFN-γ, GM-CSF, G-CSF, M-CSF, anti-CTLA-4, Bcl-2 antisense, vitamin A, anti-CD19, anti-CD20, anti-CD22, anti-CD28 or anti-CD3.

20

412. A method for displaying recombinant molecules, which molecules include a native or engineered immunoglobulin heavy chain variable region, the improvement comprising a immunoglobulin heavy chain region that includes one or more mutation, substitution, alteration, and/or deletion at one or more amino acid residue
25 corresponding positions 9, 10, 11, 12, 108, 110, and 112 in said heavy chain variable region.

413. A non-naturally occurring single chain antigen-binding protein comprising protein having a formula selected from the group consisting of 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3, 2H7 scFv VH L11S IgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WlgACH2 T4CH3, 2H7 scFv VH
30 L11S (SSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3,

2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3, 2HU scFv VH L11S (CSS-S) H P331S
 CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH L11S
 (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q
 CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H
 5 WCH2 WCH3, G28-1 scFv IgAH WCH2 WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2
 WCH3, G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CSC-S) H
 WCH2 WCH3, G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3, CTLA4 (SSS-S) H
 P238SCH2 WCH32, CTLA4 (CCC-P) WH WCH2 WCH3, FC2-2 scFv (SSS-S) H WCH2
 WCH3, FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3, UCHL-1 scFv (SSS-S) H WCH2
 10 WCH3, UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3, 5B9 scFv (SSS-S) H WCH2
 WCH3, 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 WCH3,
 2H7 scFv (SSS-S) H P238SCH2 WCH3, 2H7 scFv IgAH WCH2 WCH3, 2H7 scFv IgAH
 WIgACH2 T4CH3, 2H7 scFv IgAH WIgACH2 WCH3 + Jchain, 2H7 scFv (CCC-P) WH
 WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 F405YCH3, 2H7 scFv (SSS-S) H WCH2
 15 F405ACH3, 2H7 scFv (SSS-S) H WCH2 Y407ACH3, 2H4 scFv (SSS-S) HWCH2 F405A,
 Y407ACH3, 2H7 scFv (CSS-S) H WCH2 WCH3, 2H7 scFv (SCS-S) H WCH2 WCH3,
 2H7 scFv (SSC-P) H WCH2 WCH3, 2H7 scFv (CSC-S) H WCH2 WCH3, 2H7 scFv (CCS-
 P) H WCH2 WCH3, 2H7 scFv (SCC-P) H WCH2 WCH3, 2H7 scFv VH L11S (SSS-S) H
 WCH2 WCH3, 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (SCS-
 20 S) H WCH2 WCH3, G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3, G28-1 scFv VH
 L11S (SCC-P) H WCH2 WCH3, G28-1 scFv VH L11S mlgE CH2 CH3 CH4, G28-1 scFv
 VH L11S mlgAH WIgACH2 T4CH3, G28-1 scFv VH L11S hlgE CH2 CH3 CH4, G28-1
 scFv VH L11S hlgAH WIgACH2 T4CH3, HD37 scFv IgAH WCH2 WCH3, HD37 scFv
 (SSS-S) H WCH2 WCH3, HD37 scFv VH L11S (SSS-S) H WCH2 WCH3, L6 scFv IgAH
 25 WCH2 WCH3, L6 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv-llama IgG1, 2H7
 scFv-llama IgG2, 2H7 scFv-llama IgG3, CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3,
 CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3, 2e12 scFv (SSS-s)H P238SCH2 WCH3—
 hCD80TM/CT, 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 40.2.36 scFv
 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2H7 scFv (SSS-s)H P238SCH2 WCH3—
 30 hCD80TM/CT, G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2e12 scFv (SSS-

s)H WCH2 WCH3—hCD80TM/CT, 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT,
 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—
 mFADD-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 2e12 scFv (SSS-
 s)H WCH2 WCH3—mcas3-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcas3-
 5 TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mcas8--TM/CT, 2e12 scFv (SSS-s)H
 P238SCH2 WCH3—mcas8-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—hcas3--
 TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcas3-TM/CT, 2e12 scFv (SSS-s)H
 WCH2 WCH3—hcas8--TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcas8-
 TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 1D8 scFv--hIgG1
 10 (SSS-s)H WCH2 WCH3—hCD80TM/CT, 1D8 scFv—mIgAT4—hCD80TM/CT, 1D8
 scFv--hIgE—hCD80TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-
 TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 1D8 scFv--hIgG1
 (SSS-s)H WCH2 WCH3—mcas3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2
 WCH3—mcas3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcas8--TM/CT,
 15 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcas8-TM/CT, 1D8 scFv--hIgG1 (SSS-
 s)H WCH2 WCH3—hcas3--TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—
 hcas3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcas8--TM/CT, 1D8 scFv--
 hIgG1 (SSS-s)H P238SCH2 WCH3—hcas8-TM/CT, L6 scFv (SSS-S) H WCH2 WCH3,
 2H7 scFv CD154 (L2), 2H7 scFv CD154 (S4), CTLA4 IgAH IGACH2CH3, CTLA4 IgAH
 20 IgACH2 T4CH3, 2H7 scFv IgAH IgACH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2H&-
 40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-cd20-anti-cd40), 2H7 scFv IgAH
 IgACH2 T4CH3-hCD89 TM/CT, G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT,
 and 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT.

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